

## EFFECT OF NIMBIDIN ON GASTRIC ACID SECRETION

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**ABSTRACT:** *Nimbidin, the crude bitter compound from neem has been investigated for its gastric anti secretory activity in rats and cats. It exhibited significant anti – secretory activity in pylorus ligated rats and cats. In lumen – perfused rats it suppressed the basal as well as histamine and carbachol stimulated gastric acid output at 40 mg/kg (i.v). However it had no effect on ASA – induced back – diffusion of H<sup>+</sup> ions. This anti secretory activity of nimbidin was found to be similar to that of H<sub>2</sub> – receptor antagonists, which are reported to suppress the histamine induced gastric secretion in animals and man.*

**KEY WORDS :**

*Azadirachta indica; Nimbidin; Pylorus – ligated rats; Stomach – lumen perfused rats; Pylorus – ligated cats; H<sub>2</sub> – receptor blockade.*

### INTRODUCTION

Nimbidin, the crude bitter principle from *Azadirachta indica* A. Juss with reported antiulcer potential in experimental models<sup>1</sup> has also exhibited multiple pharmacological actions in various animal models<sup>2-4</sup>. In order to detect the mechanism of action of this potential antiulcer agent, the investigation presented in this communication was designed to study the effects of this terpenic ester on gastric acid secretion in various animals models.

### Experimental

Nimbidin used in this investigation was isolated from neem oil of the seed kernels of *A. indica* as reported earlier<sup>5</sup>. Albino rats Holtzman strain and cats, were used as

experimental animals. Nimbidin was used as 10% (v/v) colloidal solution in alcohol and the control groups received only 10% alcohol.

### In pylorus – ligated rats

Albino rats of either sex (150 – 200 g) fasted for 24h with water ad libitum were used for the study. A 4h – pylorus ligated rat preparation<sup>6,7</sup> was used to detect the anti – secretory activity of nimbidin. Test drug was administered in dose levels of 10,20,40 and 80 mg / kg orally (p.o) immediately after pyloric ligation. After surgical procedure all the animals were kept without food or water and 4 hours later, killed and stomach dissected out. The stomach was

opened and contents collected, centrifuged and volume measured. Free and total acidity of gastric contents determined by titrating against  $N / 100 \text{ NaOH}^8$ . Pepsin concentration of gastric contents was estimated by the haemoglobin method<sup>9</sup> with modifications and expressed in mg/ml of gastric fluid<sup>10</sup>. Hexosamine concentration as an index of mucin content of gastric fluid was also determined<sup>11</sup> in certain experiments.

To detect the development of tolerance on repeated dosing, nimbidin was administered orally in a daily single dose (effective dose from above test) of 40 mg / kg for 14 days. On the 14<sup>th</sup> day, pyloric ligation was performed and the final drug treatment was done intraduodenally (i.d) at the time of laparotomy. A vehicle control group receiving only distilled water was also maintained. Four hours after pyloric ligation, the rats were killed and gastric fluid collected and biochemical estimations carried out as described earlier. The data were compared with that of the acute experiments to detect tolerance, if any.

The effect of test drug on normal gastric secretion and on acid back – diffusion caused by acetyl – salicylic acid (ASA) was also studied in pylorus ligated rat preparation. Ten minutes after pyloric ligation, nimbidin (20, 40 and 80 mg / kg), ASA (100 mg/kg) or both was administered orally to the respective group of rats. All of them were killed 7 h later by an over dose of ether, gastric contents collected and its volume, acidity,  $\text{Na}^+$  and  $\text{K}^+$  concentrations were determined<sup>12</sup>.

#### **In stomach – lumen perfused rats**

Experimental performed in adult male rats (250 – 350 g) under urethane anesthesia as per the method of Lai<sup>13</sup>. After laparotomy,

the stomach lumen was continuously perfused with 0.9% NaCl solution at 37°C at a constant rate of 0.8 ml / min and every 10 min effluent collected was titrated against  $N / 100 \text{ NaOH}$  with phenolphthalein as indicator using micropipette. The adic concentration was expressed in  $\mu\text{Eq} / 10 \text{ min}^{14}$ . Nimbidin was administered i.v. via tail vein in doses of 20 and 40 mg / kg and noted its influence on basal secretion. Gastric acid secretion was stimulated using histamine acid phosphate (250 – 500  $\mu\text{g} / 100\text{g}$ ) or carbachol (5  $\mu\text{g} / \text{kg}$ ) intravenously and influence of drug on these secretagogues was also determined. Cimetidine in doses of 100 and 200  $\mu\text{mol}/\text{kg}$  (i.v.) was used as reference drug for comparing the effect on basal gastric secretion in rats.

#### **In pylorus – ligated anaesthetized cats**

Twenty four hour fasted male cats were anaesthetized with pentobarbitone sodium (35 mg / kg i.p) and after laparotomy the pyloric end of stomach was ligated. The stomach was then washed with warm saline with a stomach tube passed through the oesophagus, 30 min later, 40 ml of 70% ethanol was given through the stomach tube. The zero hour sample was collected after 15 min and the test drug or 10% alcohol (control) was injected i.v. After 5 min histamine acid phosphate ( 2 $\mu\text{g}/\text{kg}/\text{min}$ ) was infused through the femoral vein by a slow injector. Samples of gastric contents (3 ml) were aspirated every 30 min for the next 180 min and replaced by an equivalent amount of 7% ethanol. The samples were filtered through cotton wool before determining the free and total acidity using 0,1 N  $\text{NaOH}^{15}$ . Nimbidin was given i.v. in dose levels of 20 and 40 mg/kg and 4 cats were used at each dose level.

Students ‘t’ test was employed for statistical analysis:

## RESULTS AND DISCUSSION

In all the dose levels tested nimbidin oral administration showed a decreased gastric secretory volume in 4 h – pylorus ligated rats, but without any dose – response relationship. At 40 mg/kg there was a significant fall ( $p < 0.01$ ) in gastric secretion but without any significant reduction in acidity, peptic activity or hexosamine content of gastric fluid. However at 20 mg/kg there was a significant ( $p < 0.01$ ) fall in peptic activity (Table 1).

Chronic oral dosing for 14 days did not show any significant reduction in gastric secretion or acidity but there was significant ( $p < 0.01$ ) suppression of peptic activity of gastric fluid with 40 mg / kg dose. (Table 2).

Nimbidin in all the three dose levels tested did not prevent the back – diffusion of acid ( $H^+$ ) and apparent efflux of  $Na^+$  and  $K^+$  associated with ASa treatment (Table 3).

In stomach lumen perfused rats, nimbidin in 20 and 40 mg/kg (i.v.) exhibited marked suppression of unstimulated gastric acid output (total). In 40 mg/kg treated group, the mean basal acid secretion of gastric effluent fraction was  $23.8 \pm 1.40 \mu Eq / 10$  min, whereas at the end of 160 min the acid secretion was only  $16.3 \pm 0.20 \mu Eq / 10$  min (Fig 1a and b). Cimetidine in 200  $\mu mo l / kg$  showed significant suppression of basal acid secretion with a steady fall to  $13.8 \pm 0.3 \mu Eq / 10$  min at the end of 160 min (Fig 2a and b), Nimbidin (40 mg/kg) treatment exhibited a partial suppression of the stimulatory effect of the second histamine injection (5 mg/kg) on gastric secretion in these rats (Fig 1-c). The peak acid output after the second histamine injection was  $26.3 \pm 4.0 \mu Eq / 10$  min, whereas during first injection before nimbidin was  $34 \pm 10 \mu Eq / 10$  min. Similarly the stimulatory effect of carbachol (5  $\mu g/kg$ ) was also partially blocked by nimbidin (40 mg/kg) in anaesthetised rats (Fig. 1d). The carbachol induced peak acid out after first injection was  $26 \pm 2.0 \mu Eq / 10$  min and after nimbidin the peak output of gastric acid was reduced to  $20 \pm 2.0 \mu Eq / 10$  min.

**TABLE I**

**Effect of oral administration of nimbidin on gastric secretion in pylorus – ligated rats.**

	Treatment (mg/kg)	No. of animals	Gastric secretion (mean $\pm$ S. E.)			
			Volume (ml/100g/4h)	Acidity (mEq/L)		Pepsin Conc. (mg/ml)
				Free	Total	
a.	Vehicle – Control	12	$3.61 \pm 0.32$	$67.66 \pm 7.45$	$124.75 \pm 9.25$	$159.66 \pm 5.93$
b	Nimbidin – 10	10	$3.31 \pm 0.28$	$68.20 \pm 4.56$	$112.50 \pm 3.70$	$145.40 \pm 5.49$
c	Nimbidin – 20	12	$2.65 \pm 0.37$	$49.25 \pm 7.89$	$105.25 \pm 6.44$	$132.33 \pm 5.74^{**}$

d	Nimbidin – 40	10	2.23 ± 0.21**	50.90 ± 7.46	110.60 ± 12.06	141.50 ± 7.08
e	Nimbidin – 80	10	3.48 ± 0.40	81.40 ± 4.65	125.90 ± 2.42	148.40 ± 6.48

P value - \*\* < 0.01

**TABLE II**

**Effect of chronic oral administration (14 days) of nimbidin on gastric secretion in rats.**

	Treatment (mg/kg)	No. of animals	Gastric secretion (mean ± S. E.)			
			Volume (ml/100g/4h)	Acidity (mEq/L)		Pepsin Conc. (mg/ml)
				Free	Total	
a.	Control – D. Water	8	4.39 ± 0.50	81.87 ± 5.80	129.75 ± 5.08	258.74 ± 16.28
b.	Vehicle – Control 10% alcohol	8	4.56 ± 0.80	80.50 ± 5.98	130.75 ± 5.33	230.16 ± 17.03
c.	Nimbidin – 40	12	3.55 ± 0.41	81.75 ± 6.80	139.16 ± 3.42	177.41 ± ** 3.65
d.	Nimbidin – 80	8	3.42 ± 0.64	85.00 ± 7.63	130.00 ± 7.63	228.80 ± 2.82

P value - \*\* < 0.01

**TABLE III**

**Effect of nimbidin, asaa and nimbidin + asa on gastric secretion in pylorus – ligated**

	Treatment	Dose mg/kg	No. of rats	Gastric contents			
				Volume (ml/100g/7h)	H+	Na+ (mEq/L)	K+
a.	Control (1 % CMC)	-	10	6.6 ± 0.62	94.1 ± 3.92	77.4 ± 5.57	7.1 ± 0.52
b.	Vehicle Control (10 % alcohol)	-	10	6.8 ± 0.41	92.7 ± 4.23	75.9 ± 5.44	7.3 ± 0.56
c.	Nimbidin	20	10	5.9 ± 0.72	105.3 ± 8.65	75.8 ± 5.44	6.3 ± 0.87

d.	Nimbidin	40	10	5.3 ± 0.57	92.5 ± 2.75	88.4 ± 6.47	7.8 ± 0.81
e.	Nimbidin	80	8	6.2 ± 0.06	86.5 ± 2.75	62.5 ± 8.05	9.1 ± 1.25
f.	ASA	100	10	5.4 ± 0.38	80.2 ± 3.98	83.6 ± 6.12	8.4 ± 0.61
g.	Nimbidin + ASA	20 + 100	10	6.6 ± 0.82	79.0 ± 5.65	77.8 ± 5.54	9.1 ± 0.84
h.	Nimbidin + ASA	40 + 100	10	6.4 ± 0.57	77.7 ± 5.04	76.9 ± 3.59	7.7 ± 0.74
i.	Nimbidin + ASA	80 + 100	10	6.3 ± 0.57	78.5 ± 4.34	62.5 ± 12.68	9.3 ± 0.24

P Values – H + : b:e N. S., b : f < 0.05, b : g N. S.

b : h < 0.05, b : I ( 0.05, f : h N. S.

Volume - b : c N. S., b : d < 0.05, b : f < 0.05

In anaesthetized cats, nimbidin 40 mg/kg (i.v.) showed an apparent reduction in both free and total acidity of gastric effluents under constant histamine infusion. At 120 min of drug administration there was a significant ( $p < 0.05$ ) reduction in free acidity. However test drug in 20 mg / kg dose did not show any effect on histamine induced gastric acidity but it rather increased the acidity. But the gastric acidity induced by histamine was neither completely blocked nor brought back to basal level during the period of observation (Table 4).

Histamine H<sub>2</sub> – receptor antagonists inhibit not only gastric secretion elicited by injected histamine, but also basal secretion and that elicited by various physiological stimuli<sup>16</sup>. Even though nimbidin did not exhibit and H<sub>2</sub> – receptor blocking activity in rat uterus (in – vitro study) (unpublished data), in stomach lumen perfused rat preparation, test drug was found to inhibit basal acid

secretion in both dose levels and at 40mg/kg it markedly reduced the histamine and carbachol stimulated acid output. Similarly, nimbidin in 40 mg/kg dose level significantly reduced the free acidity in anaesthetized cats receiving a constant i.v. infusion of histamine. Cimetidine, the potent H<sub>2</sub> – receptor antagonist also caused inhibition of basal as well as histamine evoked gastric secretion in rats and dogs<sup>17</sup> and in man a similar dose – dependent effect is seen after oral or parenteral administration<sup>18</sup>. Hence the anti – secretory effect of nimbidin in cats and rats, could be attributable to its pasted H<sub>2</sub> – receptor antagonism. Thus the anti – ulcer potential of this compound might be due to its anti secretory property contributed by its H<sub>2</sub> – receptor antagonism as well as weak anti cholinergic and ganglion blocking properties already reported.

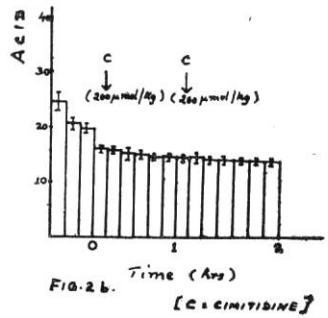
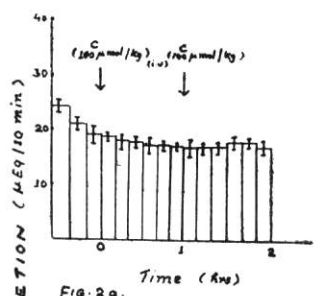
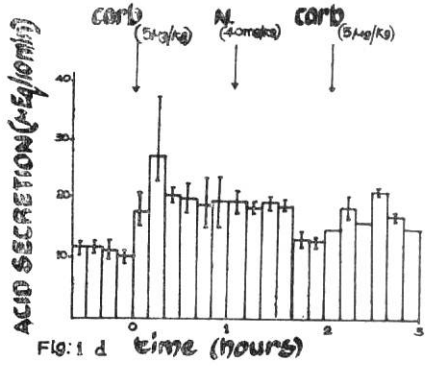
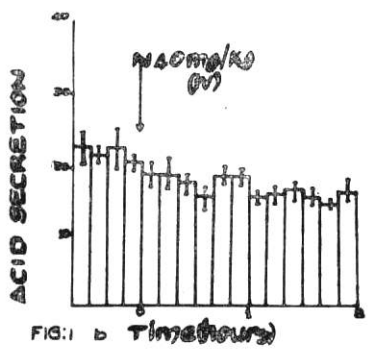
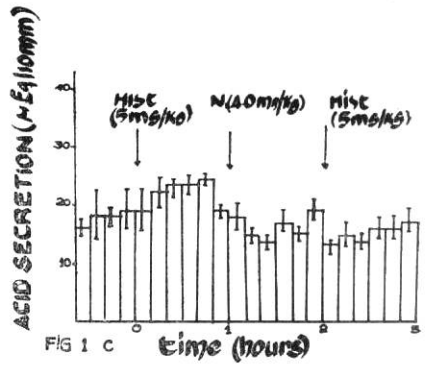
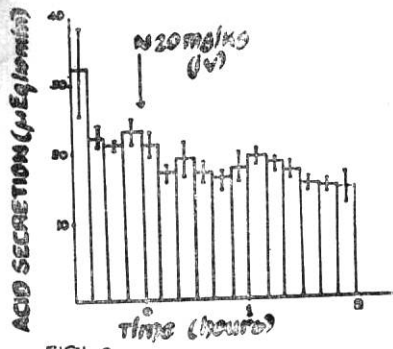


Figure Legends

- Fig. 1 - (a & b). Effect of Nimbidin on gastric acid secretion in stomach lumen perfused anaesthetized rats. Basal secretion noted for first 40 min and then given the test drug.
- a. Nimbidin 20mg / kg (i. v.) on unstimulated gastric secretion.
  - b. Nimbidin 40mg / kg (i. v.) on unstimulated gastric secretion.
- Fig. 1 - (c & d). Nimbidin on gastric acid secretion in stomach lumen perfused anaesthetized rats. Basal secretion collected for first 40min and then Secretagogues given.
- c. Nimbidin 40mg / kg (i. v.) on histamine (5mg/kg) stimulated gastric secretion.
  - d. Nimbidin (40g / kg) on carbachol ( $\mu\text{g}/\text{kg}$ ) stimulated gastric secretion.
- Fig. 2 - (a & b). Effect of cimetidine 100 and 200  $\mu\text{mol}/\text{kg}$ (i. v.) on unstimulated gastric acid secretion in rats.

**TABLE IV**

**Effect of nimbidin on histamine ( 2 µg/kg/min) induced gastric acidity in anesthetized cats**

Treatment (mg/kg i.v.)	0 min		30 min		40 min		90 min		120 min		150 min		180 min		
	FA	TA	FA	TA	FA	TA	FA	TA	FA	TA	FA	TA	FA	TA	
(Mean ± S. E)															
i. Vehicle – control (10% Al.) (4)	0	7.5 ± 2.3	4.0 ± 2.0	11.4 ± 3.9	8.7 ± 3.5	19.6 ± 9.3	15.5 ± 4.1	23.7 ± 6.2	25.3 ± 4.1	33.4 ± 5.8	24.4 ± 6.5	35.7 ± 10.3	40.5 ± 7.5	51.6 ± 9.5	
ii. Nimbidin – 20 (4)	0	8.0 ± 4.2	6.6 ± 2.7	17.3 ± 2.7	12.6 ± 6.2	22.8 ± 6.4	22.4 ± 11.3	37.1 ± 17.8	35.4 ± 17.4	58.2 ± 28.2	36.4 ± 18.6	50.9 ± 22.8	39.7 ± 0.1	54.3 ± 24.1	
iii. Nimbidin – 40 (4)	0.8 ± 0.2	6.2 ± 0.1	1.9 ± 1.5	6.8 ± 2.8	5.1 ± 3.1	10.4 ± 4.5	7.6 ± 3.5	14.4 ± 5.9	11.5 ±* 4.1	22.5 ± 6.2	14.7 ±* 0.1	25.1 ± 5.4	18.5 ±* 5.4	32.8 ± 0.1	

P value - \* < 0.05. Figures in the parenthesis indicate number of animals in the group.

FA - Free acidity, TA - Total acidity.

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